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Progress report

Experimental ulcerative disease of the colon in animals

Until recent years, there have been few successful attempts to produce experimental ulcerative disease of the colon. Several methods, including the injection of cholinergic and adrenergic drugs, the prolonged administration of histamine and histamine-releasers, the local injection of collagenase, the oral or intraarterial administration of lysozyme, as well as immunological procedures, have been used to produce experimental 'colitis' in various animal species, but the significance of these reactions in relation to the clinical condition in man remains in doubt[1]. Of considerable interest from the immunological aspect is the work of Zweibaum, Morard, and Halpern[2]. They immunized rats with living *E. coli* injected subcutaneously in Freund's adjuvant and produced a form of haemorrhagic ulcerative 'colitis', which they considered as being very similar to human ulcerative colitis in its symptoms, course, and histological characteristics.

Four years ago, it was reported that extracts of various red seaweeds fed to animals in their drinking water caused ulcerative disease of the colon in several species, including guinea-pigs, rabbits, rats, amd mice[3]. The extracts contained carrageenan, a sulphated polysaccharide of high molecular weight (100000-800000)[4]. Mild acid hydrolysis of the native carrageenan produced a degraded product with a molecular weight of less than 30000 but retaining its original sulphate content and polyanionic properties. The degraded product was found to be more ulcerogenic than the native or undegraded carrageenan. These observations have subsequently been confirmed in guinea-pigs and rabbits[5,6]. The most recent work indicates that other high molecular weight sulphated products such as sulphated amylopectin and sodium lignosulphonate cause similar lesions in the colon of animals[7]. A new field has thus been opened up in the experimental investigation of ulcerative disease of the colon[8].

Carrageenan-induced Ulceration

Carrageenan-induced ulceration of the colon in small laboratory animals was first recognized in guinea-pigs which had been fed 1% aqueous extracts of the dried red seaweed, *Eucheuma spinosum*, over a period of five months. Although all of the animals had gained weight satisfactorily, when the animals were sacrificed at the end of the experiment, careful examination of the colon using transmitted light revealed the presence of multiple ulcerations[9]. The same extract was found to cause ulceration of the colon in rabbits when fed in the drinking water over a period of three months.

In further experiments using carrageenan derived from the same seaweed but degraded by mild acid hydrolysis ulcerative lesions of a similar kind were produced not only in guinea-pigs and rabbits but also in mice and rats.

The lesions in mice were found only on histological examination; in rats they were recognized macroscopically by the presence of strictures[10]. The pathology of carrageenan-induced ulceration has been more fully investigated in the guinea-pig and rabbit, the two species in which lesions can be produced in a relatively short period of time.

GUINEA-PIGS

When guinea-pigs are fed a 5% aqueous solution of degraded carrageenan, the animals lose weight and develop diarrhoea associated with visible or occult blood and mucus in the stools within three weeks[11].

Ulcers first appear in the caecum; later most of the colon and rectum are involved. The ulceration, as viewed by transmitted light, presents as clear areas (0·1 cm diameter) with a darker marginal zone of congestion. Some lesions are larger (0·4-0·5 cm diameter) and more irregular in shape, particularly in the rectum; some are linear. In the rectum, the ulcerated areas are readily seen both by transmitted and direct lighting.

Microscopically, ulceration is mainly confined to the mucosa and is associated with an acute or subacute inflammatory cellular infiltrate in the base and margins of the ulcerated areas. The infiltrate includes macrophages which in toluidine-blue-stained sections show the presence of metachromatic material (sulphated polysaccharide). Focal cellular aggregates also occur in non-ulcerated parts of the mucosa and may precede ulceration. Crypt abscesses and cystic dilatation of the glands are present, particularly in the more acute reactions. After five to six weeks, ulcers in various stages of healing and progression may be observed in the same animal. Long-term feeding experiments beyond this period have not yet been investigated.

RABBITS

As in the case of the guinea-pig, the feeding of degraded carrageenan to rabbits causes loss of weight and diarrhoea associated with visible or occult blood and mucus in the stools within three weeks[12,13]. With high concentrations (5%) of degraded carrageenan, the onset is more rapid and the course of the illness tends to be more acute and fulminating, some animals dying of intestinal bleeding within 10 to 14 days. With smaller concentrations (1%), the onset is more insidious, there is less tendency to diarrhoea, but occult blood is present in all animals by the end of three weeks; some animals may lose weight, while others fail to thrive. With low concentrations (0·1%) of degraded carrageenan fed in the drinking water, there is no diarrhoea or loss of weight, but occult blood associated with ulceration of the large bowel occurs in about 50% of animals within a period of three months.

As in the case of the guinea-pig, ulcers first appear in the caecum and later involve the more distal parts of the large bowel including the rectum. In the more acute reactions associated with high concentrations of degraded carrageenan, the ulcerated areas may be haemorrhagic or covered by a greyish-white slough and surrounded by congested, oedematous mucosa.

The transverse folds are frequently involved, the ulceration sometimes affecting the summit, sometimes the base of the fold. Within a few weeks, the folds become shortened, thickened, and indurated. In the more chronic reactions associated with low concentrations of degraded carrageenan, ulceration is less severe but affects similar sites and produces hyperplastic thickening of the mucosa both on the folds and in the haustra. Ultimately,

there may be loss of folds, severe strictures in the caecum, and hyperplastic mucosal changes accompanied by polypoidal formations[3,13].

Microscopically, the ulcerated areas show acute, subacute, or chronic inflammatory changes and ulceration may occasionally extend deeply into the submucosa. The lamina propria may be heavily infiltrated by inflammatory cells, along with foamy macrophages, and the mucosal glands may show varying degrees of hyperplasia. Crypt abscesses and cystic dilatation of the mucosal glands do occur but not to the same extent as in the guinea-pig.

Ulceration Induced by Other Sulphated Products

The discovery that sulphated polysaccharides of carrageenan type could readily induce ulceration of the colon in the experimental animal prompted investigation of other related and unrelated high molecular weight sulphated products, such as sulphated amylopectin and sodium lignosulphonate[7]. Sulphated amylopectin, with a molecular weight of several million, is a synthetic product derived from potato starch. Sodium lignosulphonate is not a polysaccharide; it is composed of sulphonated phenylpropane units, and has a molecular weight around 20000 and is a by-product in the manufacture of wood pulp from spruce trees.

As with degraded carrageenan, the feeding of sulphated amylopectin to guinea-pigs and rabbits produces a high incidence of ulcerative disease of the colon in both species, even at the 0·1 % concentration in the case of rabbits[14]. The lesions produced are macroscopically indistinguishable from those produced by sulphated polysaccharide of seaweed origin. Only minor differences exist in relation to the histological features.

Sodium lignosulphonate also produces ulcerative disease of the colon but has only been investigated in the guinea-pig. The incidence of ulceration ranges from 80 to 100% depending upon the duration of the experiment and the concentration of sodium lignosulphonate used.

Both these substances have antipeptic properties, sulphated amylopectin being marketed in the USA as Depepsen and used as a therapeutic agent in the treatment of peptic ulcer[15,16]. Lignosulphonates are incorporated in food pellets as a binding agent and used in animal feeding[17]. No adverse side effects have as yet been reported following the administration of these substances in the treatment of patients or in the feeding of animals. There is, however, much current interest in the use of antipeptic agents in the treatment of peptic ulcers[18–21]. Some of these preparations, eg, carrageenan, dextran sulphate, lignosulphonates, sodium polyanhydromannuronic acid, and sulphated amylopectin, are known to prevent or provide a measure of protection against several types of experimentally induced gastroduodenal ulcerations[22–26]. In the light of present knowledge, there is clearly a need to screen such preparations before their release for clinical use.

Relation to Human Ulcerative Colitis

From the clinical and pathological aspects, experimental ulcerative disease of the colon induced by sulphated products of high molecular weight resembles ulcerative colitis in man. Thus, the clinical pattern may vary from an acute fulminating illness to one of low-grade severity over a prolonged period.

Clinical features shared in common include weight loss, anaemia, diarrhoea, visible or occult blood, and sometimes mucus in the faeces.

From the pathological aspect, there are similarities such as ulceration of variable extent and largely confined to the mucosa, some loss of haustration due to disappearance of the horizontal folds, granularity of the mucosa along with pseudo-polyps and polypoidal formations, as well as strictures leading to intestinal obstruction. Histological features in common are acute, subacute, and chronic inflammatory changes in the mucosa, with occasional crypt abscesses, and cystic dilatation or distortion of mucosal glands, mucosal ulceration in various stages of progression and healing, and hyperplastic changes of the glandular epithelium.

While there may be many similarities between experimental ulcerative disease of the colon and human ulcerative colitis, there are also certain differences. One clinical difference is that remissions and exacerbations occur spontaneously in the human disease. In animals, this pattern can only be produced by intermittent administration of the sulphated products. A major morphological difference would appear to be the initial site of involvement of the bowel. In animals, the ulcers are first observed in the caecum and later involve the lower colon and rectum; in man, the usual sequence is rectum and lower colon first, with right-sided involvement later.

There is as yet no definite evidence that human ulcerative colitis is produced by the ingestion of sulphated polysaccharides or other sulphated products. Very soon after the initial report on the occurrence of ulcerative disease in animals fed carrageenan, Bonfils[27] stated that he had observed no instance of ulcerative colitis developing in any of 200 patients receiving degraded carrageenan in the treatment of peptic ulcers. In countries in which red seaweeds are included in the diet, eg, Ireland, Japan, South Pacific islands, there is no indication that the incidence of ulcerative colitis differs from that in other countries. It is highly significant however, that ulcerative disease of the colon can be caused by the ingestion of sulphated products of high molecular weight in at least four different animal species.

Experimental Model

Irrespective of the relationship of the experimentally induced lesions to human ulcerative colitis, these animal experiments in which various sulphated products are fed in the drinking water provide a simple model for the investigation of ulcerative disease of the colon[28].

The results already obtained from the use of this model have opened up interesting possibilities with regard to factors concerned in the pathogenesis of ulceration of the colon.

These sulphated products have in common the ability to induce ulcerative disease, the morphology of which is more or less similar in any one species. Dose-response studies in rabbits indicate that with increasing dosage of degraded carrageenan or sulphated amylopectin, there is an increased incidence and extent of ulceration of the colon[12,14]. This would suggest that these sulphated preparations or their degraded products may be directly responsible for the damage to the colon. Although differing in chemical composition, they possess in common high molecular weight and sulphate radicles which impart polyanionic properties. It is possible, therefore, that

the powerful electronegativity of these polyanions is the common factor which accounts for their ulcerogenic properties.

Using this experimental model, one can investigate, not only factors concerned in pathogenesis, but also the secondary effects of ulceration, eg, liver changes, effects on protein metabolism, electrolytic changes in the cellular and extracellular spaces, as well as other systemic complications. It may also be used to study the influence of drugs on the pathogenesis and course of the ulcerative disease process.

J. WATT
R. MARCUS
Department of Pathology,
University of Liverpool;
Surgical Unit,
Clatterbridge Hospital,
Bebington, Cheshire.

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